

OPTIMIZATION OF THE BIOMASS PRODUCTION FROM SPROUTS OF NEO-TROPICAL VEGETABLE SPECIES

Juan Fabricio **Lazcano-Escobar**¹, Nayeli Sarahí **Quiñones-Islas**^{1,2},
Sergio Rubén **Trejo-Estrada**¹, Carolina **Ramírez-López**^{1*}

¹ Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada, (CIBA-IPN). Ex-Hacienda San Juan Molino, Carretera Estatal Tecuexcomac-Tepetitla 1 km 1.5, Tlaxcala, México. C. P. 90700.

² Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Tecomán. Carretera Colima-Manzanillo km 35, Tecomán, Colima, México. C. P. 28100.

* Corresponding author: caramirezl@ipn.mx

ABSTRACT

Seed germination improves the nutritional quality of plant foods; since they increase the contents and availability of essential nutrients and reduce the levels of antinutrients. A considerable number of species have been studied, but to date, there are few studies on vegetable species of the neo-tropics. The aim of this study was the evaluation and optimization of mineral nutrient solutions [Adelusi-Oseni (AO), Ruakura (Ru), Maeda (M)] and their combinations with compost extracts (EC1, EC2, and EC3) obtained from three different solvents, on the development of biomass of four neo-tropical vegetable plants in Mexico: pumpkin (*Cucurbita pepo* L.), papalo [*Porophyllum ruderale* (Jacq.) Cass.], pipitza (*Porophyllum linaria* Cav.), and purslane (*Portulaca oleracea* L.). The hypothesis was that biomass production achieved with the nutrient solutions would lead to the highest yield for each seed compared to the control. Germination was carried out in Petri dishes with cotton pads as substrate, adding 5 mL of nutrient solution, along with a control grown with water, in a photoperiod of 12 h; continuing germination until completing 96 h. San Cristóbal experimental design was applied to the nutrient mixtures with the greatest positive impact on the production of biomass. An ANOVA was carried out with Tukey and Dunnet means comparison tests. For pumpkin sprouts, solution EC3E2 managed to increase seedling growth by 395.2 % in comparison to the control; EC2A displayed a greater elongation in comparison with the control of 84.7 % for papal sprouts; EC2A produced an increase of 15.8 % in pipitza. In purslane, EC2A produced an increase of 53.7 %. Nutrition with compost extracts can favour the production of biomass from sprouts.

Keywords: *Cucurbita pepo* L., *Porophyllum ruderale* (Jacq.) Cass., *Porophyllum linaria* Cav., *Portulaca oleracea* L., nutrient solutions.

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INTRODUCTION

Food production systems must be renewed to generate products with better nutritional attributes. Seed sprouts contain high levels of nutrients, as a consequence of the bioavailability of ions and biomolecules derived from physiological changes during the germination and initial growth process (Liu *et al.*, 2019; Zhang *et al.*, 2020).

The germination process consists of a series of biochemical and physiological changes that result in the initial growth stages of a new plant. This process implies the mobilization of reserve substances inside the seed so the growing embryo uses those (Lynch *et al.*, 2012). Therefore, sprouts are a source of proteins, vitamins, and minerals, as well as glucosinolates, phenolic compounds, and compounds that contain selenium and isoflavones that aid in keeping good health (Ponce de León *et al.*, 2013).

Neotropical vegetables have a high content of vitamins, antioxidants, and dietary fibre, and the lack of lipids in their composition gives them a minor calorie content (Vera-Sánchez *et al.*, 2016). In addition, they contain phytochemicals such as phenols and carotenoids, which make them functional foods (Johns and Maundu, 2006).

Pumpkin (*Cucurbita pepo* L.) is an annual plant with a creeping habit that produces a fruit that is normally consumed before reaching maturity. Seeds exhibit antidiabetic, antifungal, antibacterial, anti-inflammatory, and antioxidant properties (Rolnik and Olas, 2020). The germination of seeds improves their nutritional quality and causes an important change in biochemical and sensorial characteristics (Liu *et al.*, 2019). Germination reduces the factors responsible for flatulence, increasing digestibility, and the use of proteins and carbohydrates (Akintade *et al.*, 2019).

Papalo [*Porophyllum ruderale* (Jacq.) Cass.] is an herbaceous annual plant that produces edible leaves that are consumed fresh; if they were cooked, they would lose their original flavour. Papalo is also a part of the traditional medicine of original Mexican cultures (Castro-Lara *et al.*, 2018).

Pipitza (*Porophyllum linaria* Cav.) is an annual plant that produces flowers and aromatic and flavouring leaves. This species also displays anti-inflammatory and medicinal properties (Vázquez-Atanacio *et al.*, 2021). It is rich in vitamins B and C, and it provides minerals such as phosphorous, iron, and calcium (Castro-Lara *et al.*, 2018). It is mainly used as a fresh or dry spice in different regional dishes.

Purslane (*Portulaca oleracea* L.) is an herbaceous creeping plant with soft sprouts that are added to salads as vegetables or cooked. (Castro-Lara *et al.*, 2018). This species provides vitamins A, B, and C and essential minerals such as iron, magnesium, and calcium. Purslane also contains terpenes, phenolic acid, coumarins, flavonoids, and alkaloids such as oleoresins (Baradaran *et al.*, 2019), with antifungal, antiviral, neuroprotective, and anticancer effects (Meng *et al.*, 2016).

Fourteen essential elements have been defined for crop nutrition, including the macronutrients N, P, K, Ca, Mg, and S, along with the micronutrients Cl, Fe, B, Mn, Zn, Cu, Mo, and Ni. These elements can be provided to the plants via fertilizers in the soil in open-air systems, or via the nutrient solutions in hydroponic systems (Lynch *et al.*, 2012). In addition, plant nutrition may improve by contributing organic materials such as humic substances. These substances come from plant and animal wastes; they are acidic, with high molecular weights, resistant to microbial attacks, and with refractory properties (Pepper and Gentry, 2015). They have positive effects on the growth, development, and production of crops (Hayes, 1991).

Germination helps to improve nutritional values and reduces the antinutritional and undigestible factors of seeds. Additionally, germination helps increase the synthesis

and accumulation of secondary metabolites, such as vitamin C and polyphenols, which may have antioxidant, antidiabetic, and anticancer effects. The aim of applying this evaluation method to sprouts of the four species is to identify the nutrient solutions that best ensure the greatest production of biomass. The San Cristóbal design has been used to evaluate the influence of fertilizers on the yield of various crops (Myers *et al.*, 2009). The objective of applying this evaluation methodology to the sprouts of the four vegetable species is to identify the best nutritional solutions that ensure the highest biomass production. Based on this, a biomass production system was evaluated by obtaining sprouts from four species of neotropical vegetables, to optimize and evaluate the effect of nutrient solutions [Adelusi-Oseni (AO), Ruakura (Ru), and Maeda (M)], compost extracts (EC1, EC2, and EC3), and the combinations of these.

MATERIALS AND METHODS

Biological material used

This study used commercially available seeds of four neotropical species of vegetables, traditional to Mexico: pumpkin (*Cucurbita pepo* L.), papalo [*Porophyllum ruderale* (Jacq.) Cass.], pipitza (*Porophyllum linaria* Cav.) and purslane (*Portulaca oleracea* L.), acquired from local producers of the area in the San Andrés Cholula market, in Puebla, Mexico.

Disinfection of seeds

Seeds were disinfected following the protocol described by García *et al.* (2008). First, 50 g of seeds were washed in Tween 80 (0.25 % v/v) for 5 min. Next, the seeds were soaked in 70% ethanol for 1 min, followed by a wash in 5 % Ca(ClO)₂ plus Tween 80 (0.25 % v/v) for 20 min. Finally, they were rinsed four times for 2 min each in sterile distilled water over a drain to remove excess water.

Seed sprout

Once the seeds were cleaned and disinfected, the sprouting process began based on the method described by Chen and Chang (2015), with some modifications. The seeds were soaked in purified water for 24 h and later, ten seeds were placed as an experimental unit in each Petri dish on cotton fabric as a means of support, adding 5 mL of nutrient solution, along with control purified water, applying a 12 h photoperiod in the plant growth chamber, adjusted to a temperature of 25 °C, with a light source (SEV brand, Puebla, Mexico). Each trial was evaluated in triplicate. The sprout system was rehydrated every 24 h with 2 mL of their respective nutrient solution for 96 h, at the end of which the lengths of the sprout were determined, both in the aboveground section and the root.

Nutrient solutions and organic amendments

In each one of the three species studied, the effect of the nutrient solutions was evaluated: 1. Adelusi-Oseni (AO): used for the accumulation of photosynthetic pigments (Adelusi and Oseni, 2015); 2. Ruakura (Ru): used to increase the content of

dry matter in sprouts (Smith *et al.*1983); and 3. Maeda (M): reported to increase the yields of sprouts (Maeda, 2006).

In addition, three compost extracts were tested: compost extract 1 (EC1), compost extract 2 (EC2), and compost extract 3 (EC3) with three solvents used to extract the bio-stimulants present in the composts: detergent (D), water (A) and ethanol (E). The latter factor (ethanol) was tested at three different concentrations: 25 % (1), 50 % (2) and 75 % (3) (Table 1).

Experimental design and statistical analysis

To individually evaluate the use of compost extracts and mineral nutrient solutions, an analysis was performed on the treatments carried out on the four species studied using a randomized experimental design with three repetitions of 18 experiments plus a water control of each 10-seed experimental unit (Table 1), adding a total of 228 experimental runs. An analysis of variance (ANOVA) was carried out, and the means were compared using Dunnett's test ($p \leq 0.05$) to know if there were significant differences with the control, and Tukey's means comparison test ($p \leq 0.05$) to validate the difference between the sprouts developed with the different nutrient solutions. For these analyses, the software Microsoft Excel version 16.29 and Minitab version 18 were

Table 1. The experimental design used in seed germination.

Nutrient solution	Compost extract	Treatment number
EC1 [†]	D ^{¶¶}	1
	A ^{§§}	2
	E1 ^{bb}	3
	E2 ^{mm}	4
	E3 ⁺⁺⁺	5
EC2 [¶]	D	6
	A	7
	E1	8
	E2	9
	E3	10
EC3 [§]	D	11
	A	12
	E1	13
	E2	14
	E3	15
M ^p	-	16
AO ^a	-	17
Ru ⁺⁺	-	18
Testigo	-	19

[†]EC1= Compost extract 1, [¶]EC2= Compost extract 2, [§]EC3= Compost extract 3, ^pM= Maeda nutrient solution, ^aAO= Adelusi Oseni nutrient solution, ⁺⁺Ru= Ruakura nutrient solution, ^{¶¶}D=Detergent, ^{§§}A=Water, ^{bb}E1= 25 % ethanol solution; ^{mm}E2= 50% ethanol solution, ⁺⁺⁺E3= 75 % ethanol solution.

used. Pareto charts were used to identify the nutrient solutions that generated greater biomass in sprouts.

The San Cristóbal experimental design was then used to generate a second experiment to determine the treatments needed to value the effect of the interaction between mineral solutions and compost extracts on the length of the sprouts of the four species evaluated, compared to a control with water. This test was carried out based on the program Minitab 18, applying a compound central design with a triplicated repetition in the central point (Table 2).

RESULTS AND DISCUSSION

Germination tests for the selection of nutrient media

Figure 1 shows the difference in percentages in terms of the elongation of the aerial section of the pumpkin sprouts (subfigure 1A), papalo (subfigure 1B), pipitza (subfigure 1C), and purslane (subfigure 1D) in regard to the control (sprouts germinated using only purified water), according to the treatments described in Table 1.

Figure 1A shows that the greatest development of the pumpkin sprout was in EC3E2, a treatment in which a length of 61.90 ± 15.37 mm was reached, equivalent to an increase of 395.2% concerning the control, followed by the AO mineral solution, with a length of 43.44 ± 12.08 mm for the sprout, equivalent to an increase of 250% regarding the control, and by the compost extract named EC1A, with a length of 37.50 ± 12.32 mm, equivalent to a 200% increase regarding the control.

Table 2. Experimental design applying the San Cristóbal experimental design of the individual solutions that generated greater biomass of sprouts to evaluate the mixture of nutrient solutions in the production of sprouts of the seeds studied.

Treatment number	Nutrient solutions
1	EC2A [†] -AO [‡]
2	EC3E2 [§] -AO
3	EC2A-Ru [¶]
4	EC3E2-Ru
5	EC2A
6	EC3E2
7	AO
8	Ru
9	EC2A-EC3E2
10	Ru-AO
11	(EC3E2-Ru)-(EC2A-AO)
12	(EC2A-EC3E2)-(Ru-AO)
13	(EC3E2-AO)-(EC2A-Ru)

[†]EC2A: compost extract 2 extracted with water, [‡]AO: Adelusi Oseni nutrient solution, [§]EC3E2: compost extract 3 extracted with 50 % ethanol solution, [¶]Ru: Ruakura nutrient solution.

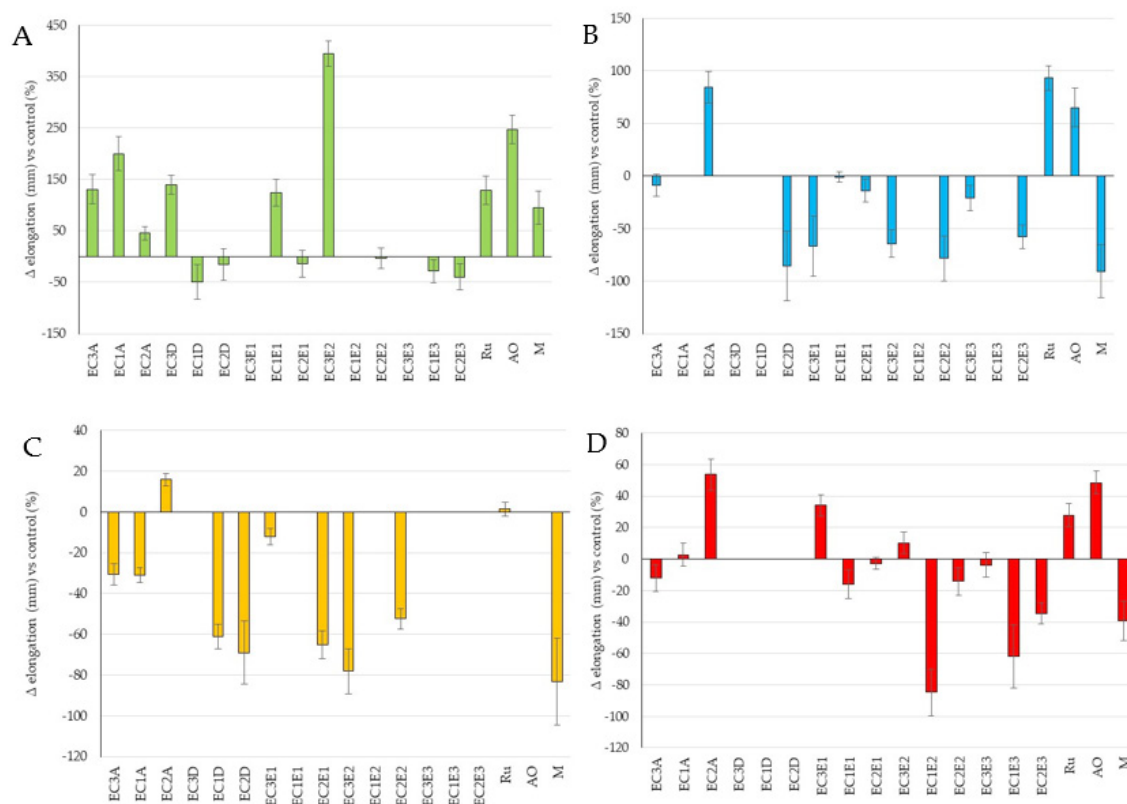


Figure 1. Percentage of difference in sprouts elongation: A: pumpkin; B: papalo; C: pipitza and D: purslane in comparison with the control sprout.

Regarding papalo, the solutions that recorded the greatest developments in the aerial section were the Ru mineral solution, with 41.04 ± 4.79 mm, which is equal to an increase of 93.2% in comparison with the control; treatment EC2A, with an aerial section measuring 39.24 ± 5.91 mm, equal to an increase of 84.7% in comparison with the control, and the AO solution, which developed 35.12 ± 6.38 mm, which is 65.3% more than the control. In the rest of the experiment, no values of much importance were observed in terms of the development of the sprout, and a lack of viability in seeds was displayed, which is why some data are not shown or a 0% is shown, considering that they are identical to the control.

The results in pipitza are shown in Figure 1C. Compost extract EC2A can be observed to have produced an elongation of 9.66 ± 3.18 mm, which represents an increase of 15.8% in comparison with the control. This was the only nutrient solution that presented a greater development in the aerial section of the sprout in pipitza seeds in comparison with the control. The other treatments reduced this variable in pipitza.

The results for purslane are shown in Figure 1D. Treatment EC2A, with a sprout length of 20.20 ± 4.41 mm, equivalent to an increase of 53.7% in comparison with the control; in AO with a length of 19.53 ± 2.38 mm and an increase of 48.6% in comparison

with the control, and EC3E1 with a length of 17.64 ± 3.79 mm and an increase of 34.2% in comparison with the control, all showed the greatest growth of the aboveground section for this species.

The evaluation of the effect of the compost extracts in the sprout and elongation of sprouts showed that each vegetable evaluated has specific requirements for its development. Therefore, the choice of nutrient solutions with the highest positive influence on the sprouts were compost extracts EC2A and EC3E2, as well as mineral solutions Ru and AO.

Based on the above results, a second experimental design was proposed (Table 2), applying the San Cristóbal design with triplicate repetition in the central point in the program Minitab 18, using individual nutrient solutions and mixtures with which a greater impact was observed on the growth of the aerial section of the pumpkin, papalo, pipitza and purslane sprouts, compared to a control with water. A total of 13 treatments were carried out in triplicate, of which those that developed sprouts with greater length were selected.

The results of this second germination are shown in Figure 2. The effect of the different treatments on the elongation of the sprouts can be seen, and they are compared with the lengths of the sprouts developed using water (controls).

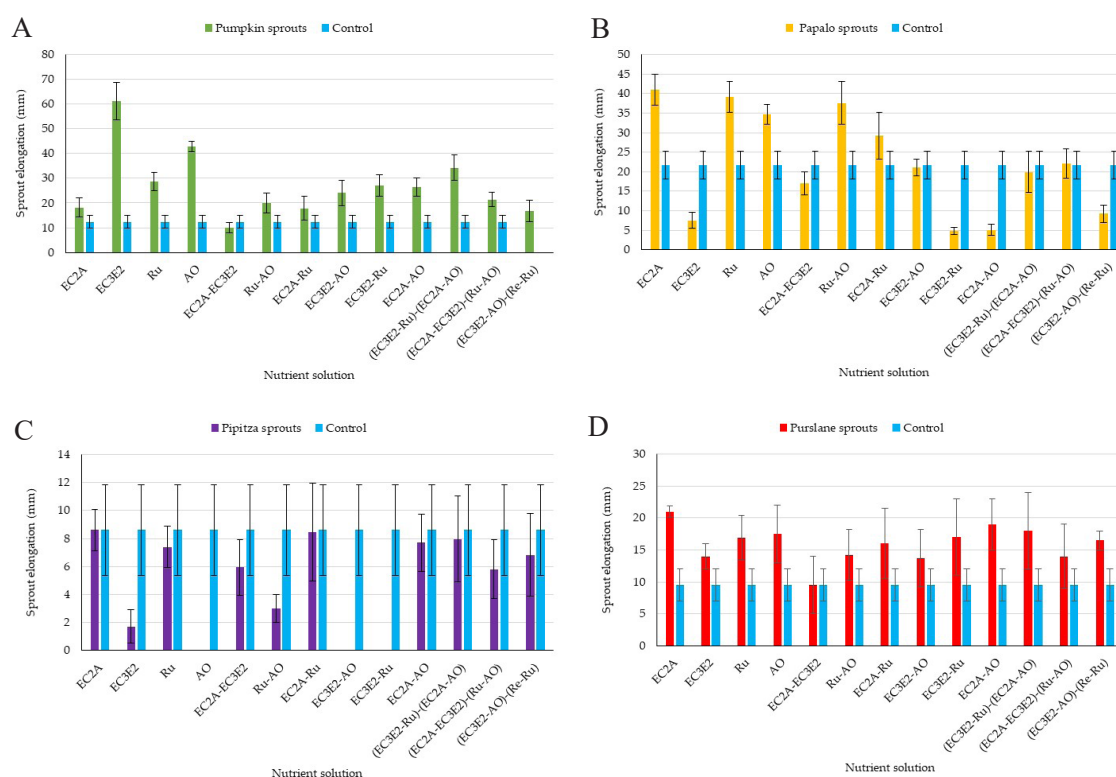


Figure 2. Sprout elongation (mm) in seeds: A: pumpkin; B: papalo; C: pipitza and D: purslane, compared with the control.

In pumpkin, the greatest length of the aerial section of the sprout was achieved in treatment EC3E2, with a length of 61.20 ± 7.57 mm, and in AO, with 42.83 ± 2.06 mm in length (Figure 2A). Dunnett's test (Table 3) indicates significant differences between the arithmetic mean of the lengths achieved with these solutions regarding the control, whereas Tukey's test (Table 3) indicates significant differences between the means of the lengths between EC3E2 and AO. This may be explained because the humic substances can form complexes with the transition metal cations, which may increase the absorption of these elements by the plant (Chen and Aviad, 1990). In addition, plants treated with humic substances have a higher water absorption efficiency than untreated plants, which they can use to produce more biomass. A similar study on basil (*Ocimum basilicum*), cilantro (*Coriandrum sativum*), and lettuce (*Lactuca sativa*)

Table 3. Tukey ($p \leq 0.05$) and Dunnett tests ($p \leq 0.05$) to compare the arithmetical means of the length of the sprouts (mm) of pumpkin, papalo, pipitza, and purslane sprouts.

Nutrient solution	Pumpkin	Papalo	Pipitza	Purslane
Control	12.33 ± 2.52 E, F [†] ; A [‡]	21.75 ± 3.53 C, D [†] ; A [‡]	8.61 ± 3.26 A [†] ; A [‡]	9.53 ± 2.58 C [†] ; A [‡]
EC2A	18.20 ± 3.90 D, E, F [†] ; A [‡]	41.01 ± 3.88 A [†] ; - [‡]	9.28 ± 1.51 A [†] ; A [‡]	21.07 ± 2.95 A [†] ; - [‡]
EC3E2	61.20 ± 7.57 A [†] ; - [‡]	7.55 ± 1.95 F, G [†] ; - [‡]	1.77 ± 1.25 B, C [†] ; - [‡]	14.17 ± 2.02 B, C [†] ; A [‡]
Ru	28.80 ± 3.70 C, D [†] ; - [‡]	39.17 ± 4.21 A, B [†] ; - [‡]	7.44 ± 1.57 A, B [†] ; A [‡]	16.93 ± 3.14 A, B [†] ; A [‡]
AO	42.83 ± 2.06 B [†] ; - [‡]	34.63 ± 2.54 A B [†] ; - [‡]	0.00 C [†] ; - [‡]	17.55 ± 4.56 A B [†] ; A [‡]
EC2A-EC3E2	10.28 ± 2.21 F [†] ; A [‡]	17.22 ± 3.16 D, E, F [†] ; A [‡]	5.93 ± 2.13 A, B, C [†] ; A [‡]	9.47 ± 3.92 C [†] ; A [‡]
Ru-AO	20.67 ± 4.12 D, E, F [†] ; A [‡]	37.61 ± 5.48 A, B [†] ; - [‡]	3.02 ± 1.03 A, B, C [†] ; - [‡]	14.23 ± 4.02 A, B, C [†] ; A [‡]
EC2A-Ru	17.83 ± 4.85 D, E, F [†] ; A [‡]	29.26 ± 6.29 B, C [†] ; A [‡]	8.48 ± 3.50 A [†] ; A [‡]	15.93 ± 5.55 A, B, C [†] ; A [‡]
EC3E2-AO	24.07 ± 5.20 C, D, E [†] ; - [‡]	5.13 ± 1.36 G [†] ; - [‡]	0.00 C [†] ; - [‡]	13.70 ± 4.51 B, C [†] ; A [‡]
EC3E2-Ru	27.03 ± 4.35 C, D [†] ; - [‡]	4.85 ± 0.98 G [†] ; - [‡]	0.00 C [†] ; - [‡]	17.12 ± 5.43 A, B [†] ; A [‡]
EC2A-AO	26.43 ± 3.65 C, D [†] ; - [‡]	21.14 ± 2.16 C, D [†] ; A [‡]	7.70 ± 2.06 A, B [†] ; A [‡]	19.08 ± 4.17 A, B [†] ; A [‡]
(EC3E2-Ru)- (EC2A-AO)	34.17 ± 5.11 B, C [†] ; - [‡]	19.93 ± 5.22 C, D, E [†] ; A [‡]	7.97 ± 3.19 A, B [†] ; A [‡]	18.03 ± 5.82 A, B [†] ; A [‡]
(EC2A-EC3E2)- (Ru-AO)	21.37 ± 2.93 D, E, F [†] ; A [‡]	22.13 ± 3.71 C, D [†] ; A [‡]	5.80 ± 2.11 A, B, C [†] ; A [‡]	14.06 ± 4.33 B, C [†] ; A [‡]
(EC3E2-AO)- (Re-Ru)	16.87 ± 4.40 D, E, F [†] ; A [‡]	9.20 ± 2.15 E, F, G [†] ; - [‡]	6.83 ± 2.76 A, B [†] ; A [‡]	16.52 ± 1.51 A, B [†] ; A [‡]

[†]Tukey's test: Means that do not share a letter are significantly different; [‡]Dunnett's test: Means not labeled with the letter A are significantly different from the mean of the control level.

treated with compost tea found the same amount of biomass was found as in those produced with Steiner mineral solution (González-Solano *et al.*, 2018).

In papalo (Figura 2B), the greatest lengths of the aerial section of the sprouts were achieved with the compost extract EC2A, with 41.01 ± 3.88 mm. Tukey's test (Table 3) showed no significant differences regarding the Ru solution, which produced 39.17 ± 4.21 mm; the AO solution, which produced 34.63 ± 2.54 mm; or the mixture of Ru-AO solutions with 37.61 ± 5.48 mm. Meanwhile, Dunnett's test (Table 3) indicated that the sprouts developed with the treatments mentioned above are statistically superior to the control. The positive effects of the humic substances were observed primarily in plant growth indicators, such as the sprout emissions and roots, and the length or weight of fresh and dry biomass (Vaughan and Malcolm, 1985).

In pipitza (Figure 2C), Dunnett's test (Table 3) indicated no significant differences between the different treatments regarding the control, the sprout of which reached a length of 8.61 ± 3.26 mm. However, treatments EC3E2 (1.77 ± 1.25 mm) and Ru-AO (3.02 ± 1.03 mm) presented less development in their sprouts in comparison with reached with the control. In the case of treatment AO, EC3E2-AO and EC3E2-Ru, it is important to note that germination was inhibited. These results corroborate that there cannot be one single nutrient solution that produces satisfactory results in all circumstances and all species evaluated (Smith *et al.*, 1983). Nutrient solutions and humic substances may affect the synthesis and transportation of phytohormones in plants. For example, humic substances may stimulate the synthesis of indole-3-acetic acid (IAA). In turn, this auxin may stimulate the germination and growth of sprouts (Amador-Alfárez *et al.*, 2013).

In purslane (Figure 2D), the greatest development of the sprout was achieved in treatment EC2A, with 21.07 ± 2.95 mm in length. Dunnett's test (Table 3) revealed that this was the only solution that displayed a significant difference from the control. An increase in N intake, which favours the growth of the aerial section, may be explained by the role played by the humic substances in the modulation of the nitrogen intake through an interaction with the cell membrane (Maggioni *et al.*, 1987; Chen and Aviad, 1990; Gulnaz *et al.*, 1999; Verlinden *et al.*, 2010). These compounds seem to increase the permeability of the cell membrane and can have an activity similar to the phytohormones named auxins, which act as plant growth regulators (Olivares *et al.*, 2017). Small fragments of humic acids may even provide nutrients for plants. On the other hand, in nutrient solutions with negative effects or with lower sprout development stimulation abilities, their components are known for presenting chemical reactions that form ion precipitates, causing them to become unavailable for the development of the plant (Trejo-Valencia *et al.*, 2015).

CONCLUSIONS

The evaluated compost extracts are an important option to increase the biomass yield in the production of sprouts. Compost extract 3, obtained with 50% ethanol (EC3E2) increased the production of pumpkin biomass production by 395.2% in comparison

with the control. Whereas the papalo sprout with the compost extract 2, obtained with water (EC2A) produced an elongation of over 84.7% in comparison with the control. In pipitza, compost extract 2, obtained with water (EC2A) produced a biomass increase of 15.8% in comparison with the control. In purslane, compost extracts 2, extracted with water (EC2A), induced an increase of 53.7% in biomass in comparison with the control.

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